CLAIMS

1.- A DNA construct comprising:

a) a first nucleic acid sequence containing the nucleotide sequence coding for a product of interest;

b) a second nucleic acid sequence containing the nucleotide sequence coding for a dimerization domain; and

c) a third nucleic acid sequence containing the nucleotide sequence coding for *E. coli* α-hemolysin (HlyA) or for a fragment of said protein comprising the recognition signal of the *E. coli* Hly transport system secretion mechanism, or a nucleotide sequence coding for a homologous gene, or a nucleotide sequence coding for a natural or artificial variant of HlyA or of a fragment thereof comprising the recognition signal of the *E. coli* Hly transport system secretion mechanism.

2.- DNA construct according to claim 1, wherein the 3′ end of said first nucleic acid sequence is bound to the 5′ end of said second nucleic acid sequence, and the 3′ end of said second nucleic acid sequence is bound to the 5′ end of said third nucleic acid sequence.

3.- DNA construct according to claim 1, wherein said product of interest is chosen from enzymes, enzymatic inhibitors, hormones, molecules involved in cell adhesion and/or signaling, molecules involved in detection or labeling, molecules made up of domains, immunogenic antigens, therapeutic agents, and immunoregulating molecules.

4.- DNA construct according to claim 3, wherein said product of interest is chosen from tumor-specific antigens, auto-immune disease antigens, growth factors, cytokines, interleukins, interferons, and miniantibodies.

5.- DNA construct according to claim 1, wherein said dimerization domain comprises a peptide helix or a coiled coil structure.

6.- DNA construct according to claim 5, wherein said dimerization domain comprises a leucine zipper.

7.- DNA construct according to claim 5, wherein said dimerization domain comprises the leucine zipper of the yeast transcription factor GCN4.

8.- DNA construct according to claim 1, wherein said third nucleic acid sequence is chosen from:

a) the nucleotide sequence coding for HlyA of *E. coli*;

b) a nucleic acid sequence comprising the nucleotide sequence coding for the last 60 amino acids of the C-terminal end of HlyA of *E. coli*;

c) a nucleic acid sequence made up of a nucleotide sequence coding for the last 60 amino acids of the C-terminal end of HlyA of *E. coli*;

d) the nucleotide sequence identified as SEQ ID NO: 1; and

e) a nucleotide sequence coding for the amino acid sequence identified as SEQ ID NO: 2.

9. DNA construct according to claims 1 and 2, further comprising a fourth nucleic acid sequence coding for a spacer peptide located between said first and second nucleic acid sequences, wherein the 5′ end of said fourth nucleic acid sequence is bound to the 3′ end of said first nucleic acid sequence, and the 3′ end of said fourth nucleic acid sequence is bound to the 5′ end of said second nucleic acid sequence.

10.- DNA construct according to claim 9, wherein said spacer peptide comprises amino acid residue repetitions, preferably Gly-Gly-Gly-Ser repetitions.

11.- DNA construct according to claim 9, wherein said spacer polypeptide is a hinge region of an antibody.

12.- DNA construct according to claim 1, further comprising a fifth nucleic acid sequence coding for a polypeptide susceptible of being used for isolation or purification purposes.

13.- DNA construct according to claim 12, wherein said

polypeptide susceptible of being used for isolation or purification purposes comprises a polyhistidine sequence or a polypeptide sequence recognized by a monoclonal antibody and which can be useful for purifying the resulting fusion protein by immunoaffinity chromatography.

14.- DNA construct according to claim 12, wherein said fifth nucleic acid sequence is located between said second and third nucleic acid sequence ordered according to claim 2, wherein the 5' end of said fifth nucleic acid sequence is bound to the 3' end of said second nucleic acid sequence, and the 3' end of said fifth nucleic acid sequence is bound to the 5' end of said third nucleic acid sequence.

15.- DNA construct according to claim 1, further comprising a sixth nucleic acid sequence coding for a peptide susceptible of being used for recognition purposes.

16.- DNA construct according to claim 15, wherein said peptide susceptible of being used for recognition purposes comprises a peptide sequence recognized by a monoclonal antibody and can be useful for recognizing the resulting fusion protein by immunodetection techniques.

17.- DNA construct according to claim 15, wherein said peptide susceptible of being used for recognition purposes comprises the E epitope sequence.

18.- DNA construct according to claim 15, wherein said sixth nucleic acid sequence is located between said second and third nucleic acid sequences ordered according to claim 2, wherein the 5' end of said sixth nucleic acid sequence is bound to the 3' end of said second nucleic acid sequence, and the 3' end of said sixth nucleic acid sequence is bound to the 5' end of said third nucleic acid sequence.

19-. DNA construct according to claims 12 or 15, wherein said fifth and sixth nucleic acid sequences are separated from one another.

20.- DNA construct according to claims 12 or 15, wherein said fifth and sixth nucleic acid sequences are bound to one

another.

21.- DNA construct according to claim 20, wherein said fifth and sixth nucleic acid sequences are located between the second and third sequences ordered according to claim 2.

22.- DNA construct according to claim 1, further comprising a seventh nucleic acid sequence comprising a nucleic acid sequence coding for an amino acid sequence susceptible of being cleaved specifically by enzymatic or chemical means.

23.- DNA construct according to claim 22, wherein said seventh nucleic acid sequence comprises a nucleotide sequence coding for a protease recognition site.

24.- DNA construct according to claim 23, wherein said protease is chosen from an enterokinase, endoprotease Arg-C, endoprotease Glu-C, endoprotease Lys-C and coagulation factor Xa.

25.- DNA construct according to claim 22, wherein said seventh nucleic acid sequence comprises a nucleotide sequence coding for a site susceptible of being cleaved specifically by a chemical reagent.

26.- DNA construct according to claim 25, wherein said chemical reagent is cyanogen bromide.

27.- DNA construct according to claim 22, wherein said seventh nucleic acid sequence is located in any position between the second and third nucleic acid sequences ordered according to claim 2.

28.- An expression cassette comprising a DNA construct according any of the previously claims operatively bound to an expression control sequence.

29.- Expression cassette according to claim 28, wherein said expression control sequence comprises a promoter sequence, a transcriptional regulator coding sequence, a ribosome binding sequence (RBS) and/or a transcription terminating sequence.

30.- Expression cassette according to claims 28 and 29,

wherein said expression control sequence is functional in bacteria.

31.- A vector comprising at least one DNA construct according to any of claims 1 to 27 or at least one expression cassette according to any of claims 28 to 30.

32.- Vector according to claim 31, further comprising a marker.

33.- Vector according to claim 32, wherein said marker comprises an antibiotic resistance gene or a toxic compound resistance gene.

34.- A gram-negative bacteria comprising a DNA construct according to any of claims 1 to 27, or an expression cassette according to any of claims 28 to 30.

35.- A gram-negative bacteria comprising at least one DNA construct according to any of claims 1 to 27, or at least one expression cassette according to any of claims 28 to 30, or at least one vector according to any of claims 31 to 33.

36.- Bacteria according to claim 35, chosen from *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Pseudomonas putida*.

37.- A dimeric fusion protein obtainable by expression of the nucleic acid sequences contained in a DNA construct according to any of claims 1 to 27.

38.- A dimeric fusion protein obtainable by expression of the nucleic acid sequences contained in a DNA construct according to any of claims 1 to 27, or at least one expression cassette according to any of claims 28 to 30, or at least one vector according to claims 31 to 33.

39. Fusion protein according to claims 37 and 38, wherein each monomer comprises:

(i) the amino acid sequence of a product of interest.

(ii) an amino acid sequence corresponding to a dimerization domain; and

(iii) the amino acid sequence of α-hemolysin (HlyA) of *Escherichia coli* or of a fragment of said protein

comprising the recognition signal of the *E. coli* hemolysin (Hly) transport system secretion mechanism.

41.- Fusion protein according to claim 40, wherein each monomer comprises:

(i) a product of interest chosen from an enzyme, an enzymatic inhibitor, a hormone, a molecule involved in cell adhesion and/or signaling, molecules involved in detection or labeling, molecules made up of domains, an immunogenic antigen, a therapeutic agent, an immunoregulating molecule;

(ii) a dimerization domain chosen from a peptide helix and a coiled coil structure; and

(iii) the whole *E. coli* HlyA amino acid sequence, or an *E. coli* HlyA fragment comprising the recognition signal of the *E. coli* Hly transport system secretion mechanism.

41.- Fusion protein according to claim 41, wherein each monomer comprises:

(i) a product of interest chosen from a tumor-specific antigen, an auto-immune disease antigen, a growth factor, a cytokine, an interleukin, an interferon and a miniantibody;

(ii) a dimerization domain chosen from a peptide helix and a coiled coil structure; and

(iii) the whole *E. coli* HlyA amino acid sequence, or an *E. coli* HlyA fragment comprising the recognition signal of the *E. coli* Hly transport system secretion mechanism.

42.- Fusion protein according to claims 37 to 41, wherein each monomer further comprises (a) a spacer peptide between the product of interest and the dimerization domain; and/or (b) a peptide to facilitate the isolation and purification of the peptide or fusion protein; and/or (c) a

peptide which allows recognition of the peptide or fusion protein; and/or (d) an amino acid sequence susceptible of being cleaved specifically by enzymatic or chemical means.

43.- Fusion protein according to claim 42, wherein each monomer comprises (a) a peptide containing Gly-Gly-Gly-Ser repetitions or the hinge region of an antibody; and/or (b) a polyhistidine sequence or a peptide sequence recognized by a monoclonal antibody and which may be useful for purifying the resulting fusion protein by immunoaffinity chromatography; and/or (c) a peptide sequence recognized by a monoclonal antibody and which may be useful for recognizing the resulting fusion protein by immunodetection techniques; and/or (d) an amino acid sequence forming a recognition site of an enterokinase, endoprotease Arg-C, endoprotease Glu-C, endoprotease Lys-C or coagulation factor Xa, or an amino acid sequence susceptible of being cleaved specifically by a chemical reagent.

44.- A method for producing a product of interest in the form of a dimeric fusion protein according to any of claims 37 to 43, comprising growing a bacteria according to claims 34 to 36 under conditions allowing the production and excretion of said product of interest to the culture medium in the form of a dimeric fusion protein.

45.- Method according to claim 44 for producing a dimeric fusion protein, comprising two products of interest.

46.- Method according to claims 44 and 45, further comprising the isolation and purification of said dimeric fusion protein.

47.- Use of a DNA construct according to claims 1 to 27 for the creation of a dimeric protein library.

48.- Use of the library of the previous claim for choosing molecules with the capacity to bind to a given antigen.

49.- Fusion protein according to claims 37 to 43, for the use thereof in therapy.

50.- Fusion protein according to claims 37 to 43 for the use thereof in *in vitro* or *in vivo* diagnosis.